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STRUCTURE/ACTIVITY EFFECTS FOR OLIGOMERIC AND
POLYMERIC *p*-NITROPHENYL ESTERASES

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Duplication of enzymic efficiency and selectivity with synthetic materials has been the goal of much research. Studies directed toward understanding enhancement of amide and ester hydrolysis frequently utilize *p*-nitrophenyl esters of alkanolic acids, 1 as substrates to gain mechanistic insight (eq. 1). Most studies have focused on increasing efficiency through use of highly reactive catalytic sites, separately or in conjunction with covalently organized binding sites. Our goal has been to examine structure/activity relationships in linear, polymeric catalysts that self-organize in aqueous medium to form binding sites that function cooperatively with efficient catalytic groups in hydrolysis of 1.

A linear oligomer, 5 (*n* =10), recently prepared and characterized in our laboratory^{1,2}, has been evaluated as a catalyst for hydrolysis of 1. This synthetic material not only exhibits high levels of catalytic efficiency and confirms to the Michaelis-Menten model but also demonstrates enzyme-like specificity for esters derived from acids of moderate chain length (C₁₂-C₁₆) with 1 (*n*=14) the optimum

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substrate³. We now wish to report the synthesis , characterization and evaluation of 5 with different molecular weights ($n \sim 8-40$) and different backbone structures. Comparative experiments were carried out with these polymers and their low molecular weight analogs, 2-4 as catalysts for hydrolysis of 1. The influence of the molecular weight of polymers on their catalytic activity was investigated. Definition of the relationship between the structure of polymer backbone and the catalytic efficiency was investigated by replacing the "-SiOSi-" units with "(-SiO-)₄-Si-" or "-SiCH₂CH₂Si-" units (see oligomer 6 and 7).

Details on synthesis and characterizations of catalysts 1-7 will be given elsewhere⁴. Structural assignments were based on ¹H- and ²⁹Si-NMR, GPC, DIP-MS and UV data. Catalytic kinetics experiments were performed under standard conditions as reported earlier: [cat]= 5×10^{-6} M, [S]= 5×10^{-5} M, T=30 °C, methanol/ aqueous buffer (0.05 M H₂PO₄⁻/HPO₄²⁻, pH=8.0) =1:1, v/v. The pseudo-apparent first-order rate constants were obtained as slopes of plots of $\ln[A_{\infty}/(A_{\infty}-A_t)]$ vs time, where A_{∞} and A_t are the absorbances at infinite time and time t , respectively. Catalytic efficiency is taken as equivalent to the overall reaction rate constant.

Under standard conditions with 1 ($n=14$) as substrate, comparative experiments are performed among different compounds as catalysts. Increasing reaction rate constant is found in the order of $2 < 3 < 4 < 5$. This is in agreement with the prediction on increasing hydrophobicity

in catalysts based on catalyst's structures (Fig. 1). In general, the larger the alkanoic group in substituent of end-blocks, the more hydrophobic in the resulting compound. While strong hydrophobic domain forms from the self-organization of oligomers with hydrophobic backbone in aqueous medium in contrary to their low molecular weight analogs. Therefore, this result confirms that the main driving force between catalyst and substrate is derived from the hydrophobic interaction. However, by using oligomeric and polymeric catalysts with molecular weight of 2,500-13,000 in the hydrolysis of **1** ($n=14$) (eq. 1) under standard conditions, it seems apparent that in a certain range of molecular weight polymer chain's length does not significantly affect their esterase activity in view of the similar reaction rate constants. Furthermore, the optimum ratio of methanol/aqueous buffer is found remaining at 1:1, v/v in spite of a change in the molecular weight of catalysts. These phenomena imply that much aggregation in polymer coils seems not take place while the oligomer chain becomes longer in the same solvent medium.

It has been revealed that oligomer **5** exhibits remarkable substrate selectivity with **1** of $n=14$. This may be attributed to the fact that the distance between two catalytic sites of pyridine groups is equivalent to the length of alkanoic chain in substrate **1** ($n=14$). However, the alkanoic chain length for the optimum substrate is found not shifting to the substrate **1** of $n>14$ when using oligomer **6** as a catalyst under standard conditions. This is possibly due to the formation of intra-chain loops by very flexible $\text{--}(\text{--SiO--})_4\text{--Si--}$ units. Consequently, the distance between two catalytic sites remains as the same as in

oligomer 5. On the other hand, what is the role of the flexible "-SiOSi-" unit in polymer backbone? In order to answer this question oligomer 7 was prepared and its catalytic activity was measured in hydrolysis of 1 with $n=14$ under standard conditions. Oligomer 7 catalyzed hydrolysis of 1 ($n=14$) at the same rate and selectivity as oligomer 5. This unusual result indicates that oligomer's conformation remains as the same after replacement of "-SiOSi-" unit with "-SiCH₂CH₂Si-" unit in polymer backbone. Therefore, the hydrophobic interaction between catalyst and substrate seems more important than the flexibility in oligomer backbone for this esterase process.

ACKNOWLEDGEMENT

The authors gratefully appreciate support for the work from the Office of Naval Research.

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